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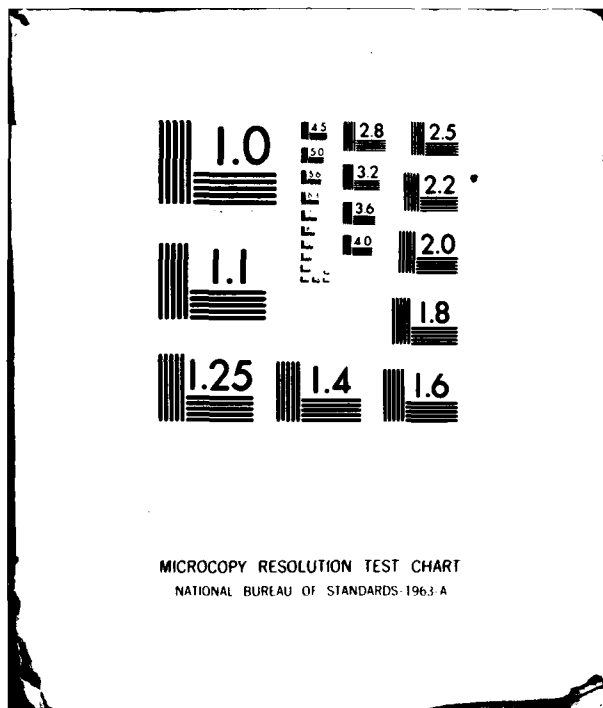
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Legionella pneumophila and TATLOCK Bacterium: Simple, Effective
Giemsa Stain

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ABSTRACT

A Giemsa stain technique for demonstrating L. pneumophila and Legionella-like organisms in culture or tissue impression smears and scrapings is described which is simple, rapid and effective. It is recommended for routine staining of cultures, and suggested for staining impression smears and scrapings of human lung tissue samples as a potential method for early presumptive diagnosis of Legionnaires' disease and Pittsburgh pneumonia.

THE ORGANISMS which cause so called Legionnaires' disease, Pontiac fever, and Pittsburgh pneumonia are rod-shaped bacteria which are refractory to most bacteriologic and histologic stains. They are considered to be gram-negative, although they usually stain very weakly even after prolonged exposure to safranin in the gram-staining procedure. The initial successful demonstration of Legionella pneumophila in culture smears was made by McDade et al. (1) using the Giménez stain, and in human lung tissue sections by Chandler et al. (2) using a modified Dieterle silver impregnation procedure. These staining procedures are still the most widely used in studies of L. pneumophila and Legionella-like organisms. Frenkel et al. (3) reported the demonstration of L. pneumophila in infected human lung sections which were stained with Wohlbach's modification of the Giemsa stain, but noted that the May-Grünwald-Giemsa stain was unsatisfactory and that unclarified factors may interfere with good staining. Cherry et al. showed that direct fluorescent antibody staining is highly specific for detection of L. pneumophila in clinical specimens (4).

In our laboratory we have found the classic Giemsa stain to be the simplest, most rapid and useful stain for demonstrating L. pneumophila and Legionella-like organisms in smears of embryonated egg yolk-sac cultures or agar-grown cultures, and for demonstrating L. pneumophila in impression smears or scrapings of infected human and guinea pig lung tissue. Although L. pneumophila in formalin fixed, paraffin-embedded sections of infected guinea pig lungs were stained by this method, the organisms were too difficult to distinguish from tissue elements to be of practical use.

A stock solution of Giemsa's stain is prepared by adding 0.5 gm of certified Giemsa powder to 33 ml of glycerin, C.P.; the mixture is held in a 50°C water bath for several hours and shaken occasionally; finally 33 ml of absolute methanol are added and shaken thoroughly. This stock solution keeps indefinitely at room temperature. Microscope slide preparations for staining are made by gently impressing the cut surface of fresh or formalin-fixed infected tissue on slides, allowing them to air dry, and then fixing them in absolute methanol for 10 min. While fixation is proceeding, the Giemsa stain should be freshly prepared by diluting one part of stock Giemsa solution with 20 parts distilled water and mixing thoroughly. The slides should be transferred directly from the methanol fixative to the Giemsa stain, and agitated gently several times. After 20 min in the stain, the slides are rinsed gently under tap water and blotted dry with bibulous paper. Microscope slide smears of tissue scrapings, in vitro grown cultures or embryonated egg yolk-sac cultures should also be air dried, fixed in methanol and stained exactly as described above.

Giemsa-stained L. pneumophila and the TATLOCK bacterium in smears of fully grown cultures in vitro or in embryonated egg yolk-sacs were dark purple usually long, thin rod-shaped individual and diplobacilli, often arranged in parallel palisades; but short diplobacilli predominated in young cultures. In addition, long serpentine chains of bacilli were frequently present, some of which contained refractile vacuoles and did not have visible intracellular septa. Pleomorphic and vacuolated individual cells were often seen in old cultures which resembled club-shaped, vibrioform, fusiform and diphtheroid bacilli. No significant differences in bacterial morphology have been observed among in vitro or egg-grown cultures of all available isolates of the six known serotypes of L. pneumophila and of the Legionella-like TATLOCK bacterium. This technique was equally effective in demonstrating L. pneumophila in impression smears and scrapings of fresh or formalin-fixed lung tissue from a patient who died of Legionnaires' disease, and guinea pigs infected by aerosol exposure 48 h before autopsy. Masses of extracellular bacteria were readily observed in the human lung preparation, which consisted of dark purple stained individual and diplobacilli ranging in size from 1 to 4 μ , with relatively few curved and serpentine chains (Fig. 1). Histologic identification of host tissue cells was also possible with this technique. No extracellular bacteria were observed in the guinea pig lung tissue preparations, but long thin individual L. pneumophila were contained within many macrophages. Identity of L. pneumophila was routinely confirmed by direct antibody staining methods described by Cherry et al. (4).

We suggest that the morphology of L. pneumophila and the TATLOCK bacterium (5) in Giemsa stained impression smears of lung tissue from suspected cases of Legionnaires' Disease and Pittsburgh pneumonia may be

sufficiently unique to permit a presumptive diagnosis to be made. Application of the classic Giemsa stain technique for demonstrating these organisms in human tissue specimens would be appropriate as an adjunct to confirmation by direct fluorescent antibody staining and routine use for staining culture smears is recommended.

Acknowledgment

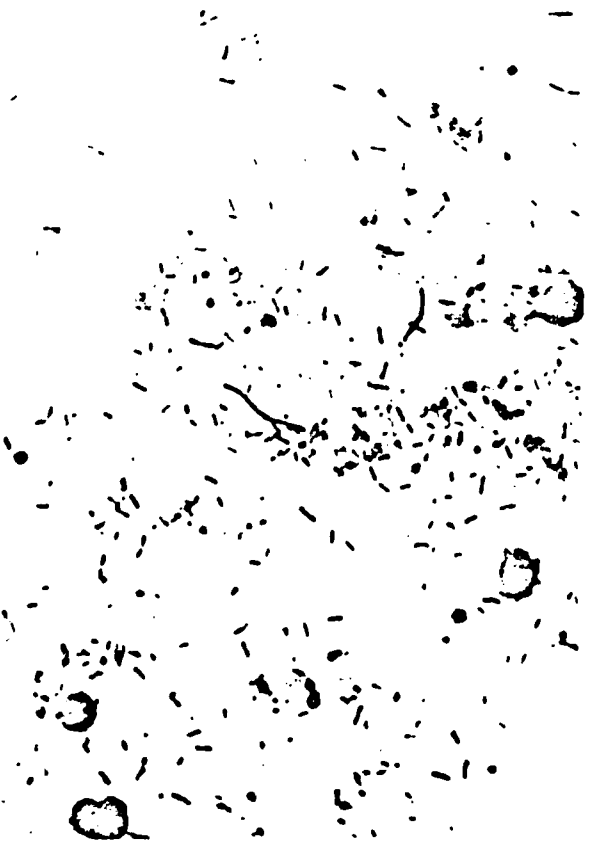
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Figure Legend

Figure 1. Giemsa stained impression smear of formalin-fixed lung tissue from a patient who died of Legionnaires' disease. Note typical variation in size of individual L. pneumophila and curved chains. (1000 X magnification).



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